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Cloning, genomic organization and chromosomal assignment of the mouse p190-B gene[1]

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Abstract

The p190 family of GTPases consists of at least two different isoforms both containing an N-terminal GTPase and a C-terminal Rho GAP domain. Here we have isolated and characterized genomic and cDNA clones spanning the entire coding region of the mouse p190-B gene. Genomic data were obtained by sequencing plasmid subclones of two overlapping mouse genomic phage clones. Interestingly, a single 3.9 kb exon was found to contain approx. 80% of the coding region of the mouse p190-B protein (amino acid residues 1–1238) including the 5′-untranslated region, the N-terminal GTPase domain and a middle domain of unknown function. Missing from this exon, however, was the C-terminal Rho GAP domain, which was cloned from mouse brain mRNA using reverse transcriptase polymerase chain reaction. Comparison of the mouse with the human p190-B proteins revealed that approx. 97% of the amino acid residues were identical. Northern analysis of total RNA from a variety of mouse tissues detected ubiquitous expression of two p190-B transcripts of 4.0 and 6.8 kb in size. Analysis of two multilocus genetic crosses localized the mouse gene, *Gfi2*, to a position on chromosome 12, consistent with the mapping of the human gene to a position of conserved synteny on chromosome 14. The high level of sequence homology between the human and the mouse suggests that there is a strong selective pressure to maintain the p190-B protein structure.

Keywords: Genomic organization; Chromosomal assignment; Mouse p190-B gene; Cloning

Members of the Ras superfamily of small GTPases play a wide variety of roles in cellular signaling mediating proliferation and differentiation, cytoskeletal organization, protein transport and secretion [1]. These proteins are active when bound to GTP and inactive when bound to GDP. The Rho subfamily of small GTPases play an important role in signal transduction pathways that regulate cytoskeletal organization [2] and some kinase signaling pathways [3]. Rho activation induces stress fibers and focal

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[1] The nucleotide sequence reported in this paper has the GenBank/EMBL Data Bank accession No. U67160.

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contacts [4], Rac promotes lamellipodia and membrane ruffling [5] and Cdc42 activates the formation of filopodia [6,7].

Proteins involved in the Rho pathway include GTPase activating proteins (GAPs), nucleotide exchange factors (GEFs), and downstream effector proteins. The GAPs for Rho family members have a conserved Rho GAP domain of approx. 150 amino acids necessary to accelerate specifically the hydrolysis of GTP bound to different Rho members [8,9]. Some of the Rho GAP proteins include BCR, p50 rho GAP, chimaerin, ABR, p122, myr-5, and two p190 proteins. Of particular interest is the p190 subfamily of Rho GAP proteins, consisting of p190-A [10] and p190-B [11]. Both proteins contain an N-terminal GTPase domain and a C-terminal Rho GAP domain. The predicted GTPase domain with the N terminus of p190-A has been shown to bind and hydrolyze GTP [12]. Recombinant proteins from the C-terminal Rho GAP domain of p190-A and p190-B accelerate the hydrolysis of GTP bound to Rho, Rac and Cdc42 consistent with their function as specific Rho GAP proteins [11,13].

Although both p190 proteins most probably play a role in the Rho pathway, additional evidence suggests that they may also be involved in other signaling pathways. For example, p190-A was originally identified as a protein that interacts with the GTPase activating protein for Ras, p120 rasGAP, suggesting that p190-A may coordinate the Rho and Ras signaling pathways [10]. Interestingly tumor suppressor activity for Ras transformation has been identified for both the GTPase and Rho GAP domains of p190-A [14]. p190-B may be involved in extracellular matrix signaling, since it has been shown that fibronectin and integrin-coated beads induce p190-B to localize adjacent to fibronectin-integrin interactions [11]. To further investigate the biological role of p190-B we have cloned the entire mouse gene, determined part of its genomic structure and mapped its chromosomal location.

A mouse 129/SvJ genomic library (Stratagene, La Jolla, CA) was screened with a ^{32}P-labeled insert of the human p190-B cDNA derived from nucleotides 1655–2734 [11]. The DNA probe was labeled by the random prime method (Boehringer Mannheim, Indianapolis, IN), purified and used in hybridization at 68°C for 18 h in 6×SSC, 5×Denhardt's reagent, 0.5% SDS and 100 μg/ml of denatured salmon sperm DNA. After washing at 68°C in 1×SSC and 0.1% SDS, the filters were air-dried and exposed to X-ray film with intensifying screens. Positive clones were obtained following three rounds of screening. Insert DNAs from two of the isolated Lambda Fix II clones (p-41 and p-42) were subcloned into the plasmid vector Bluescript SK− (Stratagene). One plasmid, HT-395, contained a 4.5 kb *Apa*I-*Not*I fragment containing part of exon 1, while the other plasmid HT-415 contained a 4.0 kb *Xba*I fragment containing part of exon 1.

The 3′-end of mouse p190-B coding for the carboxyl-terminal region of the protein was obtained by reverse transcriptase PCR using *Pfu* thermostable enzyme (Stratagene). Briefly, mouse brain cDNA was used as template with two primer adapters (*Bam*HI-*Sal*I) based on the cDNA sequence coding for amino acid residues 1213–1219 (5′-GAGGGATCCGTTGAAACTTGGAAAGGTGGT) and 1493–1499 (5′-GAGGTCGACTCGTATAATACCAAGA) of the human gene. The conditions of PCR for 30 cycles were 40 s at 94°C, 40 s at 46°C and 1 min at 72°C. Following purification and restriction enzyme digestion, the PCR product was subcloned into the *Bgl*II-*Sal*I site of the pYTH-9 plasmid vector to generate pYTH-9-mp190-B GAP. DNA sequencing revealed that this cDNA corresponded to the mouse p190-B, since it was identical with part of the sequence which overlapped exon 1 and was highly similar to human p190-B. This cDNA fragment encoding the C terminus of mouse p190-B was then labeled and used to rescreen the mouse 129/SvJ genomic library (Stratagene). Restriction enzyme analysis coupled with Southern blotting used to characterize the single positive Lambda Fix II clone.

Both oligonucleotide primers based on human cDNA sequence and mouse-derived primers were utilized. The nucleotide sequence of the genomic mouse p190-B gene was determined by dideoxy chain termination on an automated sequencer (Applied Biosystems 373A), with fluorescently labeled primers according to the cycle sequencing kit protocol as supplied by the manufacturer (Applied Biosystems). Furthermore, parts of the genomic sequences were verified by conventional sequencing using Sequenase (United States Biochemical). All database searches

were performed using the GCG version 7.0 software package.

Total RNA was prepared from mouse kidney by the method of Chomczynski and Sacchi [15]. The RNAs were subjected to electrophoresis on a 1.2% agarose formaldehyde gel, transferred to a nitrocellulose filter and probed with a random primed-labeled 2.0 kb *Bam*HI-*Bam*HI fragment (nucleotides 1370–3330) of the mouse p190-B sequence. A different fragment encoding the Rho GAP domain (nucleotides 3770–4643) was also used as probe.

The p190-B gene was mapped to a specific chromosomal position by analyzing two genetic crosses: (NFS/N or C58/J × *Mus musculus musculus*) × *M. m. musculus*, and (NFS/N × *M. spretus*) × C58/J [16]. DNAs from the progeny of these crosses have been typed for over 1200 markers which map to all 19 autosomes and the X chromosome including the Chr 12 markers *Ahr* (aromatic hydrocarbon responsiveness), *Hsp70-2* (heat shock protein 70-2), *Fv4* (Friend virus resistance 4) and *Spi1* (α_1-antitrypsin). *Aat*, *Hsp70-2* and *Ahr* were typed as previously described [17,18]. *Fv4* was typed as a *Pst*I polymorphism in the *M. m. musculus* crosses and a *Sac*I polymorphism in the *M. spretus* crosses using the 700 bp flanking fragment as probe [19]. For p190-B (*Gfi2*) a 2.0 kb *Bam*HI-*Bam*HI fragment (nucleotides 1370–3300), the same as that used for Northern analysis, was used as a probe. Mapping data were stored and analyzed using the program LOCUS developed by C.E. Buckler (NIAID, Bethesda, MD). Percent recombination and standard errors between specific loci were calculated from the number of recombinants according to Green [20]. Loci were ordered by minimizing the number of double recombinants.

A mouse genomic library was screened using a human p190-B cDNA as a probe. Ten positive clones were obtained from 1.6×10^6 independent clones. The insert DNA from two overlapping clones was subcloned into the plasmid vector Bluescript SK (−) to generate HT-395 and HT-415. DNA sequencing of these mouse genomic plasmid subclones revealed that both plasmids contained an overlapping sequence spanning a single 3884 bp exon, tentatively called exon 1 (Fig. 1). This exon coded for the N-terminal GTPase domain and an additional 900 amino acids of unknown function. Analysis of the DNA sequence of the mouse gene revealed that the nucleotide sequences surrounding the start methionine of both mouse and human p190-B genes were identical. In addition, since the mouse genomic sequence upstream from the start methionine shared high homology (90% identity over 170 bp) with the 5′-untranslated region of the human p190-B cDNA, this sequence is also highly likely to be contained within exon 1. Within this highly conserved 5′-untranslated region were some conserved direct nucleotide repeats that might be important for the stability/translational efficiency of the p190-B mRNA.

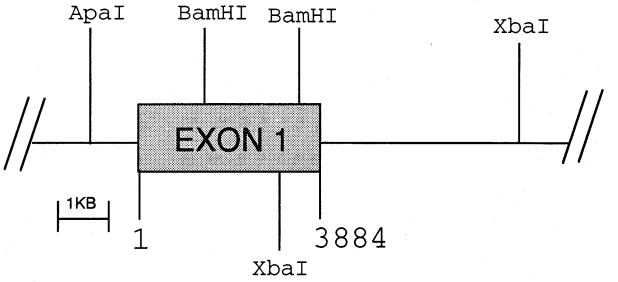

Fig. 1. Schematic organization of the mouse p190-B gene. The location of exon 1 is shown in scale with respect to several restriction sites. Restriction sites are shown for *Apa*I, *Bam*HI, and *Xba*I. The numbers for the boundaries of exon 1 were determined from DNA sequencing.

Missing from exon 1, however, was the sequence encoding the Rho GAP domain. The point of divergence was directly before the Rho GAP domain at nucleotide 3885. The nucleotides at this site contain the typical splice donor sequence AAG/gta (Fig. 2). Additional sequencing and Southern blotting with a mouse cDNA encoding the Rho GAP domain (see below) did not show the presence of any additional exons within 3 kb downstream from exon 1. In order to obtain the genomic structure of the Rho GAP domain, the same mouse 129/SvJ genomic library was rescreened using the mouse cDNA fragment from the Rho GAP domain (see below). One positive clone was obtained. Southern mapping of this clone revealed that a single 6.0 Kb *Xba*I fragment hybridized strongly with the p190-B Rho GAP cDNA probe and additional Southern blotting experiments revealed that the clone did not overlap with the two genomic cosmid clones containing exon 1 (data not shown). These results suggest that a minimal distance of at least 3 kb separates exon 1 from additional 3′ exons.

Reverse transcriptase PCR was performed using

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   1  GGAAGATGATCCACATGATCTCGAAGAAGACCTTTCTTTGGCAATGAGGGAACTACAAAGAACCAAATATAATTCTGAAATTCAGGATCT    90

  91  GTATTTTGAGATGGTTTTATTTTCAGAATGAAAAGTATATATGGTTATCTTTATGAATGTAAAGACATGAGAAAAGAGTTATGATGGCAA   180
                                                                                  M  M  A  K     4

 181  AAAACAAAGAGCCTCGACCCCCATCTTATACTGTCAGTGTAGTTGGACTCTCTGGGACTGAAAAAGACAAAGGAAACTGTGGAGTTGGAA   270
   5    N  K  E  P  R  P  P  S  Y  T  V  S  V  V  G  L  S  G  T  E  K  D  K  G  N  C  G  V  G  K    34

 271  AATCTTGTTTGTGCAATAGATTTGTACGTTCAAAAGCAGATGAATATTATCCAGAGCATACTTCTGTGCTTAGCACCATTGACTTTGGAG   360
  35    S  C  L  C  N  R  F  V  R  S  K  A  D  E  Y  Y  P  E  H  T  S  V  L  S  T  I  D  F  G  G    64

 361  GCCGAGTAGTAAACAATGATTCACTTTTATACTGGGGTGACATAACACAAAATGGTGAAGATGGTGTAGAATGCAAAATTCATGTCAATG   450
  65    R  V  V  N  N  D  S  L  L  Y  W  G  D  I  T  Q  N  G  E  D  G  V  E  C  K  I  H  V  N  E    94

 451  AACAAACAGAGTTCATTGATGACCAGACTTTCTTGCCTCATCGAAGTACAAATTTGCAACCATATATAAAACATGCAGCTGCCTCAAAAT   540
  95    Q  T  E  F  I  D  D  Q  T  F  L  P  H  R  S  T  N  L  Q  P  Y  I  K  H  A  A  A  S  K  L   124

 541  TGCAGTCAGCAGAAAAACTAATGTACATTTGTACCGATCAGCTAGGCTTGGAGCAAGACTTTGAACAGAAGCAAATGCCTGAAGGGAAAC   630
 125    Q  S  A  E  K  L  M  Y  I  C  T  D  Q  L  G  L  E  Q  D  F  E  Q  K  Q  M  P  E  G  K  L   154

 631  TCAATGTAGATGGATTTTTATTGTGTATTGATGTGAGTCAAGGATGTAATAGGAAGTTTGATGATCAACTTAAATTTGTGAATAACCTTT   720
 155    N  V  D  G  F  L  L  C  I  D  V  S  Q  G  C  N  R  K  F  D  D  Q  L  K  F  V  N  N  L  F   184

 721  TTGTCCAGCTATCAAAATCCAAAAAGCCTGTGATAATAGCAGCAACTAAATGTGATGAATGTGTGGATCATTACCTTAGAGAAGTTCAAG   810
 185    V  Q  L  S  K  S  K  K  P  V  I  I  A  A  T  K  C  D  E  C  V  D  H  Y  L  R  E  V  Q  A   214

 811  CCTTTGCCTCAAACAAAAAGAATCTTCTAGTAGTGGAAACATCAGCACGATTTAATGTCAACATTGAGACATGTTTCACTGCTTTGGTAC   900
 215    F  A  S  N  K  K  N  L  L  V  V  E  T  S  A  R  F  N  V  N  I  E  T  C  F  T  A  L  V  Q   244

 901  AAATGTTGGATAAAACTCGTGGCAAACCTAAAATTATTCCCTATCTGGATGCTTATAAAACACAGAGACAACTTGTTGTCACAGCAACAG   990
 245    M  L  D  K  T  R  G  K  P  K  I  I  P  Y  L  D  A  Y  K  T  Q  R  Q  L  V  V  T  A  T  D   274

 991  ACAAGTTTGAAAAACTTGTACAGACTGTGAGAGATTATCATGCAACTTGGAAAACTGTTAGTAATAAATTAAAAAATCATCCTGATTATG  1080
 275    K  F  E  K  L  V  Q  T  V  R  D  Y  H  A  T  W  K  T  V  S  N  K  L  K  N  H  P  D  Y  E   304

1081  AAGAATATATCAACTTAGAGGGAACAAGAAAGGCCAGAAATACATTCTCAAAGCATATAGAGCAACTCAAACAGGAACATATAAGAAAAA  1170
 305    E  Y  I  N  L  E  G  T  R  K  A  R  N  T  F  S  K  H  I  E  Q  L  K  Q  E  H  I  R  K  R   334

1171  GGAGAGAAGAATATATAAGTACCTTACCAAGGGCTTTTAATAGTGTGTTGCCAGATCTAGAAGAGATTGAACATTTGAATTGGTTGGAAG  1260
 335    R  E  E  Y  I  S  T  L  P  R  A  F  N  S  V  L  P  D  L  E  E  I  E  H  L  N  W  L  E  A   364

1261  CTTTGAAGTTAATGGAAAAGAGAGCAGATTTCCAGTTATGTTTTGTGGTGCTAGAAAAAACACCTTGGGATGAAACTGACCATATAGACA  1350
 365    L  K  L  M  E  K  R  A  D  F  Q  L  C  F  V  V  L  E  K  T  P  W  D  E  T  D  H  I  D  K   394

1351  AAATTAATGATAGGCGGATCCCATTCGACCTTCTGAGCACTTTAGAAGCAGAAAAAGTCTATCAAAAACATGTACAACATCTGATATCAG  1440
 395    I  N  D  R  R  I  P  F  D  L  L  S  T  L  E  A  E  K  V  Y  Q  K  H  V  Q  H  L  I  S  E   424

1441  AGAAAAGAAGAATAGAAATGAAGGAGAAATTCAAGAAGACTTTAGAAAAAATTCAGTTCATTTCACCTGGGCAGCCATGGGAGGAAGTTA  1530
 425    K  R  R  I  E  M  K  E  K  F  K  K  T  L  E  K  I  Q  F  I  S  P  G  Q  P  W  E  E  V  M   454

1531  TGTGTTTTGTCATGGAGGATGAAGCATTCAAATACATCACTGAGGCTGATAGCAAAGAGGTATATGGTAGGCATCAGCGAGAGATAGTAG  1620
 455    C  F  V  M  E  D  E  A  F  K  Y  I  T  E  A  D  S  K  E  V  Y  G  R  H  Q  R  E  I  V  E   484

1621  AAAAAGCCAAAGAAGAGTTTCAGGAAATGCTTTTTGAGCATTCTGAACTTTTTTATGATTTAGATCTTAATGCAACACCAAGTTCTGATA  1710
 485    K  A  K  E  E  F  Q  E  M  L  F  E  H  S  E  L  F  Y  D  L  D  L  N  A  T  P  S  S  D  K   514

1711  AAATGAGTGAAATTCATACCGTTCTAAGTGAAGAACCTAGATATAAAGCTTTACAGAAACTTGCACCTGATAGAGAATCTCTTCTACTTA  1800
 515    M  S  E  I  H  T  V  L  S  E  E  P  R  Y  K  A  L  Q  K  L  A  P  D  R  E  S  L  L  L  K   544

1801  AGCATATAGGATTTGTTTATCATCCCACTAAAGAAACATGCCTCAGTGGCCAATATTGTACAGACATTAAAGTGGAAAATTTACTTGCCA  1890
 545    H  I  G  F  V  Y  H  P  T  K  E  T  C  L  S  G  Q  Y  C  T  D  I  K  V  E  N  L  L  A  T   574

1891  CTAGTCTATTAGAGATGGATCATAACCGCGTACGCTTGTATCATGATAGTACCAATATAGACAAAGTTAATCTTTTCATTTTAGGGAAAG  1980
 575    S  L  L  E  M  D  H  N  R  V  R  L  Y  H  D  S  T  N  I  D  K  V  N  L  F  I  L  G  K  D   604

1981  ATGGCCTTGCCCAGGAACTAGCAAATGAGATAAGGACTCAATCCACTGATGATGAGTATGCCTTAGATGGAAAATTTTATGAACTTGATC  2070
 605    G  L  A  Q  E  L  A  N  E  I  R  T  Q  S  T  D  D  E  Y  A  L  D  G  K  F  Y  E  L  D  L   634

2071  TTCGGCCTGTTGATGCCAAATCGCCTTACATTTTGAGTCAGCTATGGACTGCAGCCTTTAAACCACATGGGTGCTTCTGTGTATTCAATT  2160
 635    R  P  V  D  A  K  S  P  Y  I  L  S  Q  L  W  T  A  A  F  K  P  H  G  C  F  C  V  F  N  S   664

2161  CCATCGAGTCACTGAGTTTTATTGGAGAATTTATTGGAAAAATAAGAACCGCATCTCAGATCAGAAAAGATAAATATATGACTAATCTTC  2250
 665    I  E  S  L  S  F  I  G  E  F  I  G  K  I  R  T  A  S  Q  I  R  K  D  K  Y  M  T  N  L  P   694

2251  CATTTACATTAATTCTTGCTAATCAGAGGGATTCCATTAGTAAAAATCTACCAATTCTCAGGCACCAGGGTCAGCAGTTGGCCAACAAAT  2340
 695    F  T  L  I  L  A  N  Q  R  D  S  I  S  K  N  L  P  I  L  R  H  Q  G  Q  Q  L  A  N  K  L   724

2341  TGCAGTGTCCTTTTGTAGACGTACCTACTGGTACATATCCTCGTAAATTTAATGAATCACAAATAAAGCAAGCTCTAAGAGGAGTATTGG  2430
 725    Q  C  P  F  V  D  V  P  T  G  T  Y  P  R  K  F  N  E  S  Q  I  K  Q  A  L  R  G  V  L  E   754
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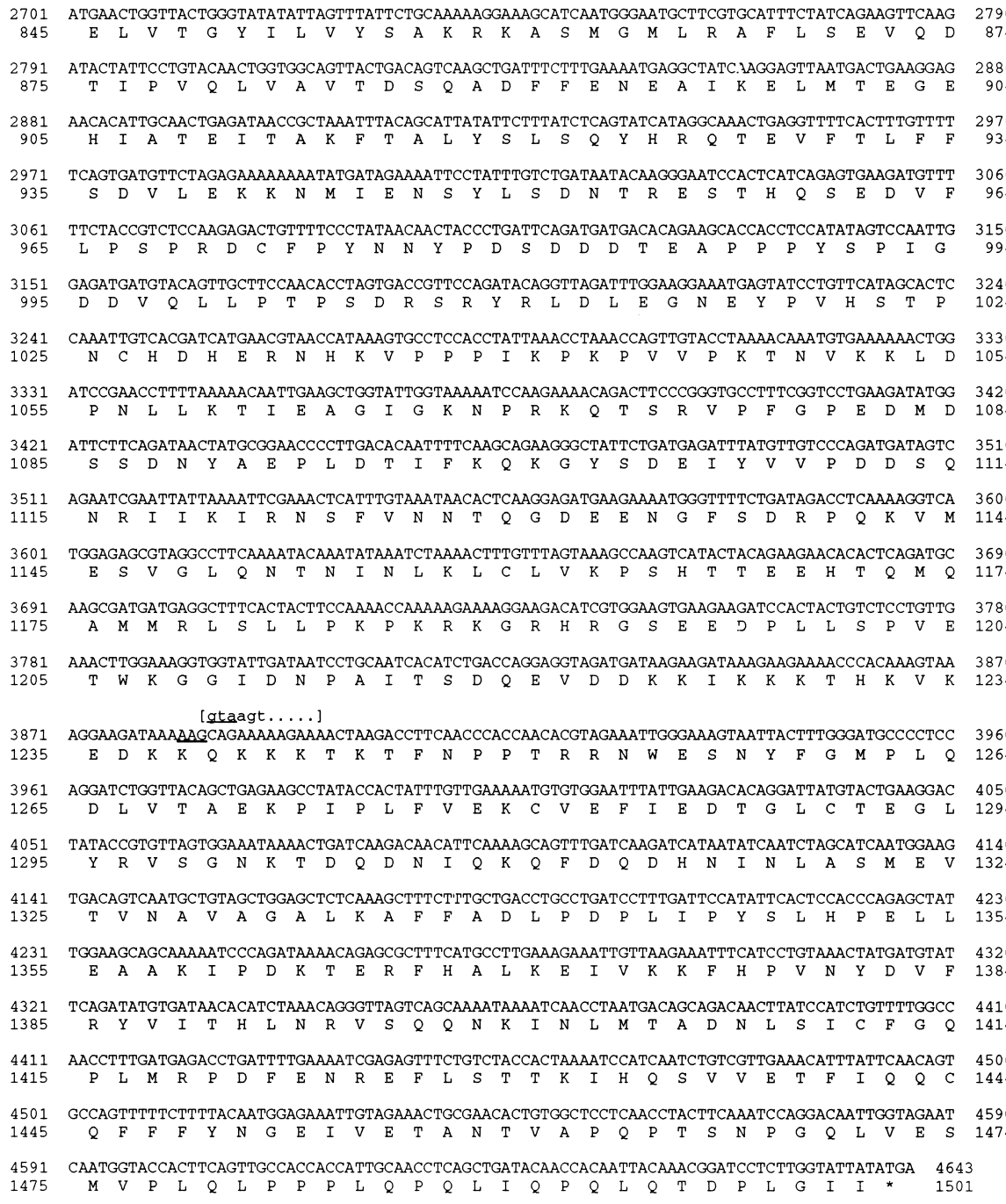

Fig. 2. Nucleotide and predicted amino acid sequences of the p190-B gene. The nucleotide and predicted amino acid sequences of the mouse p190-B gene are shown. DNA sequences were obtained from both genomic and cDNA clones. The brackets denote beginning and end of the intron sequence and the lower case letters show the DNA sequence of some of the intron.

mouse brain tissue RNA to obtain the exact nucleotide sequence of the carboxyl-terminal Rho GAP domain of the mouse p190-B. Using PCR primers based on the human sequence, a 600 bp cDNA product was generated, subcloned and sequenced. The DNA sequence obtained overlapped with the sequence from the genomic sequence of exon 1 to yield the complete coding sequence of the p190-B cDNA

(Fig. 2). Additional analysis using the GCG program BESTFIT revealed that the mouse and human p190-B nucleotide and amino acid sequences were 92% and 97% identical, respectively, spanning the entire coding sequence (data not shown). Strikingly conserved was the N-terminal GTPase domain, suggesting that this GTPase domain has a fixed structure needed for its interaction with specific effector proteins. Likewise, the Rho GAP domain was also highly conserved suggesting that this GTPase activating activity is an important function of the p190-B protein.

To examine the tissue distribution of p190-B, we performed Northern hybridization analysis. A 2.0 kb fragment derived from exon 1 was used as probe and detected two transcripts of 4.0 and 6.8 kb in a variety of mouse tissues including cerebellum, spinal cord, lung and kidney (Fig. 3). Although the 4.0 kb form was the prevalent p190-B species, in some tissues such as the testis, the 6.8 kb form is more abundant. The observation of two RNA transcripts of human p190-B has also been seen in Northern analysis using human RNA samples [11]. To determine the nature of the two mRNA species, we performed Northern analysis using a different probe derived from the C-terminal Rho GAP domain and again found hybridization with the both the 4.0 and 6.8 kb species (data not shown). These results suggest that the 4.0 and 6.8 kb RNA species differ because of alternative splicing within additional exons in the 5′- and/or 3′-flanking region.

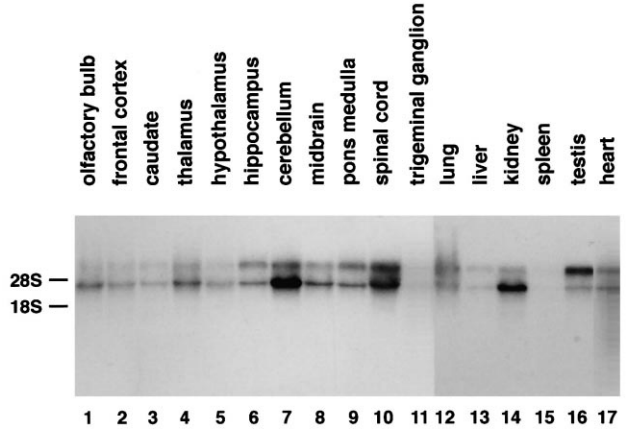

Fig. 3. Northern analysis of the mouse p190-B gene. Total RNA from mouse tissues and brain regions were analyzed for p190-B mRNA expression. The positions of the 28S and 18S ribosomal RNA are shown. Two transcripts of 4.0 and 6.8 kb were detected.

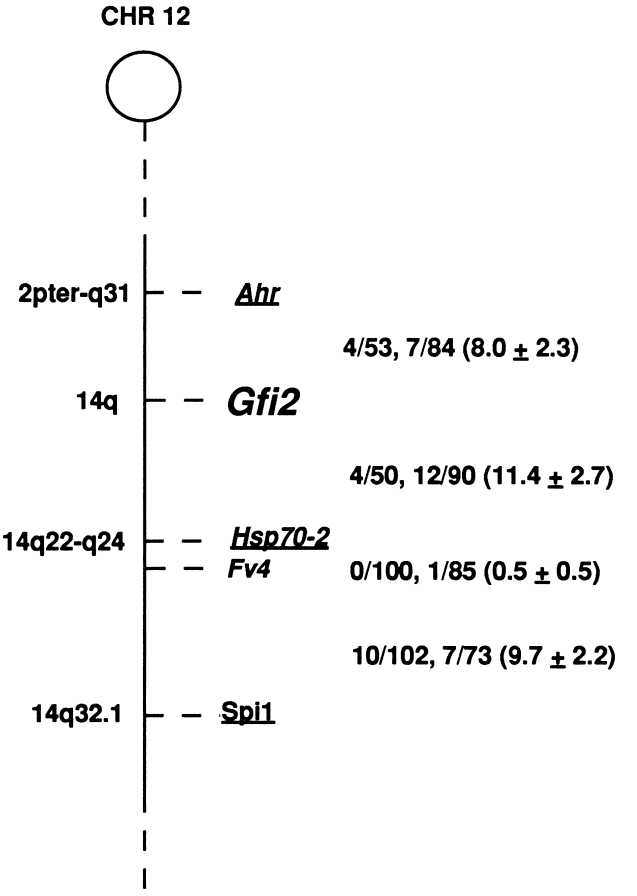

Fig. 4. Genetic map location of *Gfi2* on mouse chromosome 12. Recombination fractions are given to the right for each locus pair with the first fraction representing results from the *M. musculus* crosses and the second from the *M. spretus* crosses. Percent recombination and standard errors are given in parentheses and were determined according to Green [21]. Human map locations for homologues of the underlined genes are given to the left of the map.

The 2.0 kb *Bam*HI-*Bam*HI probe of p190-B identified *Pvu*II fragments of 9.4 kb in NFS/N and C58/J and 4.1 kb in *M. musculus* and *M. spretus*. Inheritance of these fragments demonstrated that the p190-B gene (designated *Gfi2*) mapped to Chr 12 (Fig. 4). This map location is consistent with the previously determined location of the human homologue of *Gfi2*.

In this study we describe the cloning, genomic characterization and chromosomal localization of mouse p190-B. The data described here indicate that the mouse p190-B gene is comprised of at least

two exons and is contained in a single copy on chromosome 12. The amino acid sequence of both the human and mouse p190-B predicted proteins share 97% amino acid identity, suggesting that there is great evolutionary pressure to conserve the structure of the protein.

The genomic structure of other GTPases such as Ras, Rab and Rho, show that they are encoded by several exons. The finding of a very large exon (3.9 kb) spanning 80% of the coding region of p190-B is quite surprising. The lack of an intron within this large coding region may suggest that this 1238 amino acid region may have evolved by a reverse transcription reaction of a primordial large GTPase. There are other examples of genes that have intronless coding regions including those coding for some G-protein-coupled receptors [21–23], RNase superfamily genes [24] and the interferon a1 and a2 genes [25,26]. The presence of distinct exon(s) coding for the Rho GAP domain of p190-B might suggest that this domain was added later by exon shuffling. Furthermore, the exon structure of p190-B is also unlike other Rho GAP proteins. For example, BCR, the Rho GAP gene involved in a translocation with c-Abl in leukemia, is composed of over 20 exons [27], and its Rho GAP domain spans four exons. A Rho GAP protein from *Caenorhabditis elegans*, CeGAP, is composed of at least 16 exons and its Rho GAP domain is located in only one exon [28].

Although we have mapped the p190-B gene, *Gfi2*, to chromosome 12, there appear to be no apparent mutations in this region of mouse genome. In agreement with our mouse mapping data, human p190-B has been mapped to a region of conserved synteny on chromosome 14 (Unigene collection, W14507). The regional assignment of the p190-B gene to this region suggests it may be a candidate gene for familial arrhythmogenic right ventricular cardiomyopathy [29] and a 14q deletion syndrome [30]. Mapping of the human p190-A gene to chromosome 19 (STS WI-9140; G07144) also demonstrates that human p190-A and p190-B are located on different chromosomes. It will be of interest to see whether the p190-A gene isoform shows a similar genomic organization to that of p190-B.

The role of p190-B still remains to be investigated. The multi-domain nature of p190-B and other Rho-GAP proteins supports the notion that these proteins play a role in downstream signaling. For example, the p122 Rho GAP protein interacts with phospholipase D [31] and myr-5 Rho GAP protein binds to actin [32]. The Rho GAP protein, oligophrenin-1, is mutated in some forms of non-specific X-linked mental retardation (MRX), further demonstrating the important role of Rho GAP proteins in neuronal development [33]. One approach in determining the function of a gene is to generate a null mutation that can be introduced into the germline of mice, allowing the observation of the corresponding phenotype [34]. For example, mice lacking p120rasGAP die before birth and show endothelial disorganization of the vascular system and extensive neuronal cell death suggesting a potential involvement of this GAP protein in these activities during embryonic development [35]. Similarly, the molecular cloning of genomic clones for p190-B should allow its targeted disruption in mice to yield further information about the function of this large, unusual GTPase.

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